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An efficient synthesis of 7α,12α-dihydroxy-4-cholesten-3-one and its biological precursor 7α-hydroxy-4-cholesten-3-one: Key intermediates in bile acid biosynthesis ☆

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ABSTRACT

This paper describes a method for the chemical synthesis of 7α,12α-dihydroxy-4-cholesten-3-one (**1a**) and its biological precursor, 7α-hydroxy-4-cholesten-3-one (**1b**), both of which are key intermediates in the major pathway of bile acid biosynthesis from cholesterol. The principal reactions involved were (1) building of the cholesterol (*iso*-octane) side chain by 3-carbon elongation of the cholane (*iso*-pentane) one, (2) oxidation sequence to transform the 3α-hydroxy group of the steroidal A/B-ring to the desired 4-en-3-one system, and (3) appropriate protection strategy for hydroxy groups in the positions at C-7 and C-12 in the steroid nucleus. The absolute structure of **1a** and **1b** were confirmed by NMR and X-ray crystallography. The targeted compounds **1a** and **1b**, prepared in 11 steps from **2a** and **2b** respectively, should be useful for biochemical studies of bile acid biosynthesis or clinical studies of bile acid metabolism, as plasma levels of **1b** (also termed C4) have been shown to correlate highly with the rate of bile acid biosynthesis in man.

1. Introduction

Bile acids are synthesized by the liver from cholesterol by a complex series of reactions involving at least 14 enzymatic steps [1a,1b]. In humans, a failure of any of these enzymatic reactions will result in accumulation of intermediates in the bile acid biosynthesis pathway, as well as a deficiency in the normal primary bile acids, cholic acid (CA; **2a**) and chenodeoxycholic acid (CDCA; **2b**). Defective bile acid biosynthesis presents clinically as liver disease in infants and may be fatal unless treated by bile acid replacement therapy; such therapy is life-saving [2,3].

As shown in Fig. 1, the initial biosynthetic transformation of cholesterol to the primary bile acids (**2a** and **2b**) involves sequential 7α-hydroxylation (catalyzed by cholesterol 7α-hydroxylase, *Cyp7A1*) followed by C-3 oxidation and concomitant double bond migration (catalyzed by a C_{27} 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase, *HSD3B7*) to form 7α-hydroxy-4-cholesten-3-one (**1b**), which is a precursor of CDCA (**2b**) [1c]. The transformation of **1a** catalyzed by the microsomal steroid 12α-hydroxylase *Cyp8b1* leads to the formation of 7α,12α-dihydroxy-4-cholesten-3-one (**1a**), which is ultimately converted to CA (**2a**) [1d].

The two unsaturated oxysterols (**1a** and **1b**) are thus pivotal biosynthetic intermediates in the so-called "neutral" pathway of primary bile acid biosynthesis and are of sustained interest in the biosynthetic, biological, physiological, and metabolic studies of bile acids [3]. In addition, the plasma level of **1b** has been shown to correlate highly with the rate of bile acid biosynthesis [4], and is thus a useful non-invasive biomarker for detecting increased bile acid biosynthesis. Such a determination is useful in patients with idiopathic diarrhea to determine whether bile acid malabsorption is present, as the compensatory increase in bile acid synthesis may lead to chronic diarrhea, because of bile acid induced secretion in the large intestine [5].

For the accurate analysis of **1a** and **1b** in plasma, authentic reference compounds are needed. Although **1b** is available from

Abbreviations: *CYP7A1*, cholesterol 7α-hydroxylase; *HSD3B7*, 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase; TEMPO, 2,2,6,6-tetramethylpiperidine 1-oxyl free radical; IBX, 2-iodoxybenzoic acid.

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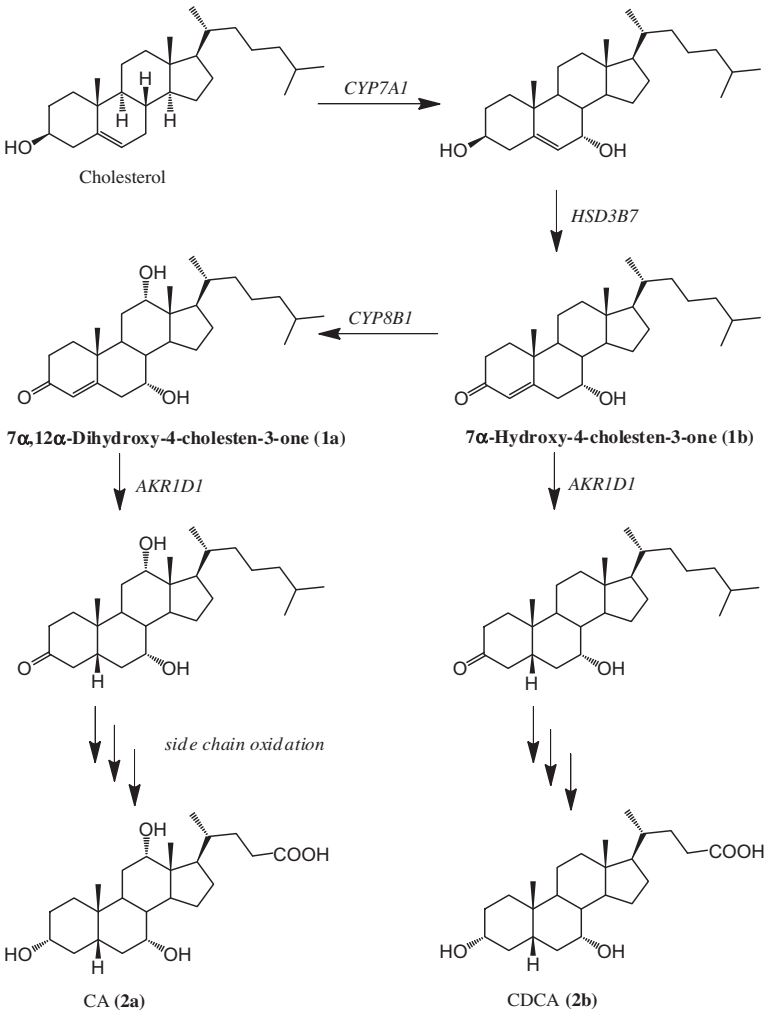


CYP7A1: Cholesterol 7α-hydroxylase/ *HSD3B7*: 3β-Hydroxy-Δ^5-C_{27}-steroid oxidoreductase
CYP8B1: Sterol 12α-hydroxylase/ *AKR1D1*: Δ^4-3-Ketosteroid 5β-reductase

Fig. 1. Major biosynthetic pathway from cholesterol of the two primary bile acids in man – CA (**2a**) and CDCA (**2b**).

commercial sources, it is extremely expensive. **1a** is not marketed. To our knowledge, however, the chemical synthesis of **1a** and **1b**, which have a Δ^4-3-oxo-7α-hydroxy structure in the steroid nucleus and the *iso*-octane (cholestane) side chain at the C-17 position, has not yet been reported. During the course of our studies on bile acid metabolism in vertebrates, we required **1a** and **1b** as authentic reference standards. We report here the chemical synthesis of **1a** and **1b** using inexpensive reagents and readily available steroid sources. Very recently, Björkhem have reviewed his five decades with oxysterols, particularly for **1b** [Biochimie 2013;95:448-54].

2. Results and discussion

2.1. Enzymatic synthesis

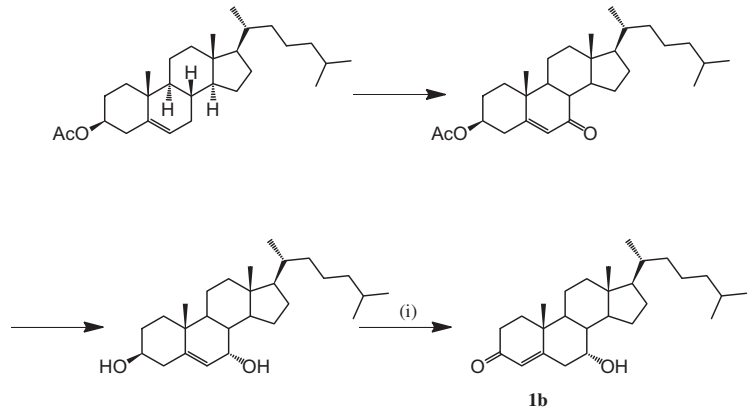
Alexander and Fisher reported some years ago a convenient enzymatic synthesis of 7α-hydroxy-4-cholesten-3-one (**1b**) by the hydroxypropyl-β-cyclodextrin-facilitated cholesterol oxidase oxidation of 5-cholestene-3β,7α-diol (7α-hydroxycholesterol) [6]. In this enzymatic synthesis using cholesterol oxidase (from *Brevibacterium* sp.) and catalase, there is a simultaneous oxidation of the 3β-hydroxy group to the 3-oxo group and migration of the double bond from Δ^5 to Δ^4; this procedure has been used for the preparation of Δ^4-3-oxo-7α-hydroxy steroids [7]. In preliminary work, we developed an improved enzymatic method for the preparation of **1b** and its 7β-isomer, by the route shown in Fig. 2. However, such an enzymatic synthesis is expensive, does not yield a pure product, and is useful only on a micromolar scale. Furthermore, a major shortcoming of this enzymatic synthesis is its inability to generate 7α,12α-dihydroxy-4-cholesten-3-one (**1a**), the metabolic precursor of cholic acid, a major primary bile acid in vertebrates.

Thus, we described herein an alternative, non-enzymatic chemical preparation of **1a** and **1b**, starting from easily available commercial materials.

2.2. Chemical synthesis

We report here synthesis of **1a**, the target compound, from **2a** in 11 steps, according to the route shown in Fig. 3. The principal steps consisted of (1) the elongation (by three carbon atoms) of the C_5 cholane (*iso*-pentane) side-chain in **2a** to the C_8 cholestane (*iso*-octane) side chain in five steps and (2) the transformation of the A/B-ring structure into the 3-oxo-Δ^4-7α,12α-dihydroxy steroid nucleus in six steps.

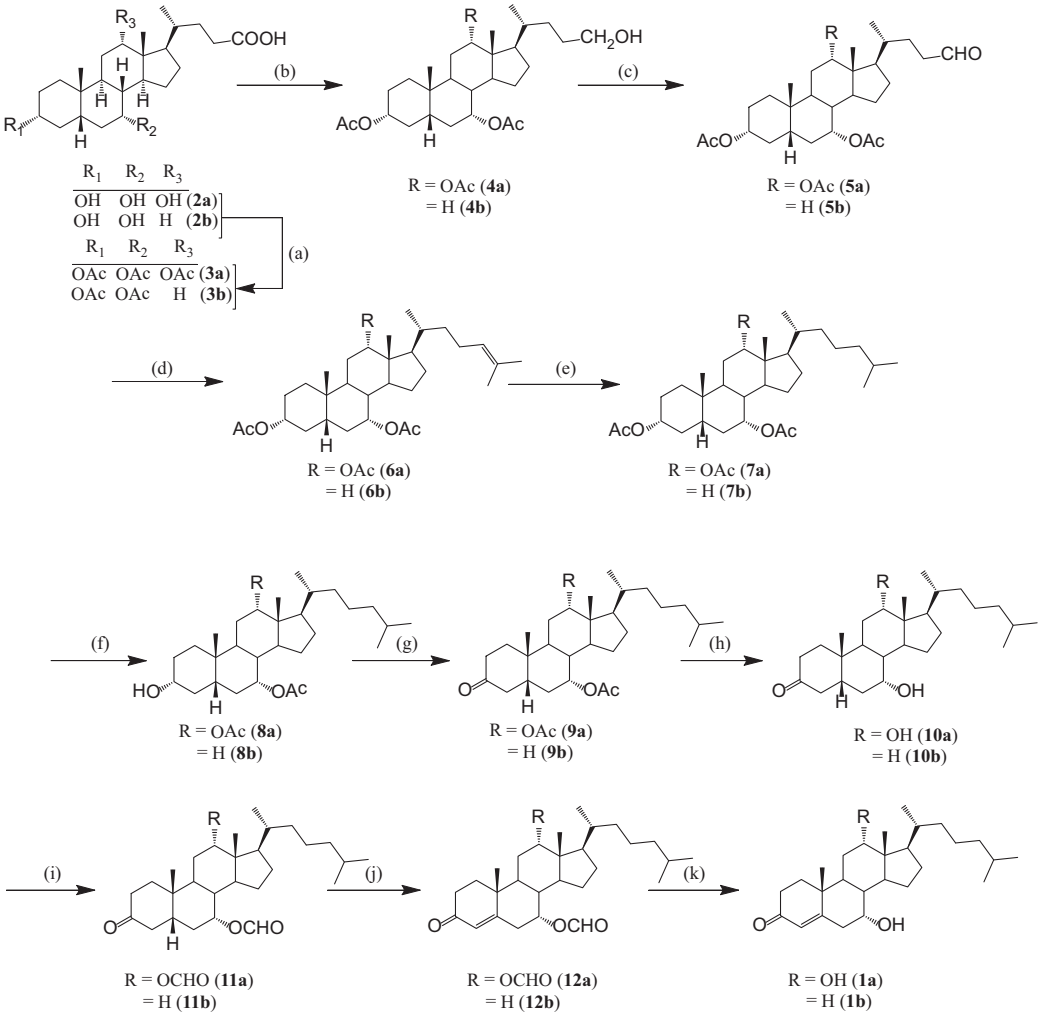
Our initial effort was directed to the construction of the cholestane side-chain from **2a**. Reduction of the terminal carboxyl group to the corresponding primary alcohol is usually carried out with



1b

Reagents and conditions: (i) hydropropyl-β-cyclodextrin/ cholesterol oxidase/ catalase/ phosphate buffer pH 7/H_2O, at 20 °C for 24 h.

Fig. 2. Enzymatic synthesis of **1b** from cholesterol.



Reagents and conditions: (a) acetic anhydride/ 4-dimethylaminopyridine/ pyridine, at 40 °C for 1 h. (b) $NaBH_4$/ Et_3N/ ethyl chloroformate/ THF, at 0 °C for 2 h. (c) 2,2,6,6-tetramethylpiperidine 1-oxyl free radical/ KBr/ NaOCl aq./ Na_2CO_3 aq./ $NaHCO_3$ aq. buffer (pH 8.6), at 0 °C for 20 min. (d) isopropyltriphenylphosphonium iodide/ 1.6M *n*-BuLi in hexane/ THF, at 0 °C for 30 min, then tris[2-(2-methoxyethoxy)ethyl]amine/ THF, at 0 °C for 15 min. (e) H_2/ 10% Pd/C/ MeOH:AcOH: EtOAc (10:10:1), at r.t. for 3 h. (f) HCl/ MeOH, at 30 °C for 6 h. (g) CrO_3/ H_2SO_4/ acetone/ CH_2Cl_2, at 0 °C for 30 min. (h) 10% KOH/ MeOH, at r.t. 16 h. (i) HCOOH/ $HClO_4$, at r.t. for 1 h. (j) IBX/ trifluoroacetic acid/ $DMSO_3$ at 40 °C for 40 h. (k) 0.2N NaOH aq., at r.t. 30 min.

Fig. 3. Synthetic route to the target compounds (**1a** and **1b**) from bile acids (**2a** and **2b**).

$LiAlH_4$ under strong alkaline conditions. However, this reduction technique has a disadvantage, as it also causes partial or complete hydrolysis of the acetyl protecting groups at C-3, C-7 and C-12 in **3a**. Therefore, we explored the use of less basic $NaBH_4$, combined with ethyl chloroformate and triethylamine (Et_3N) [8]. Thus, CA peracetate, **3a**, prepared from **2a** by the usual method, was treated with $NaBH_4$/ethyl chloroformate/Et_3N in dry THF to give the corresponding 24-hydroxy-3α,7α,12α-triacetate **4a**. As expected, the reduction reaction proceeded smoothly *via* the anhydride derivative of **3a** as an intermediate without hydrolysis of the acetyl groups.

Preliminary experiments revealed that the conversion of **4a** to the corresponding 3α,7α,12α-triacetoxy-24-aldehyde (**5a**) by the use of pyridinium chlorochromate (PCC) and pyridinium dichromate (PDC) as oxidants was unsatisfactory; the yield was less than 60% and required tedious purification of the desired product by chromatography. However, when **4a** was subjected to oxidation with sodium hypochlorite ($NaClO{\cdot}nH_2O$) in the presence of catalytic amounts of 2,2,6,6-tetramethylpiperidine1-oxyl (as free radical; TEMPO) in a $NaHCO_3$ buffer solution [9], mild, selective oxidation of the C-24 primary alcohol **4a** took place, to afford the corresponding C-24 aldehyde **5a** in good isolated yield of 79%.

Subsequent Wittig reaction of **5a** with isopropylphenylphosphonium iodide in the presence of *n*-BuLi at 0 °C for 15 min, yielded the unsaturated Δ^{24}-3,7,12-triacetate **6a** having the C_8 branched side chain. Although the basic conditions resulted concurrently in partial or complete deprotection of the acetyl groups at C-7 and C-12 in **6a**, these mixtures were re-acetylated without difficulty. Catalytic hydrogenation of **6a** using 10% Palladium on carbon (Pd/C) catalyst in a solvent mixture of EtOAc–methanol–acetic acid under slightly positive pressure proceeded smoothly to afford 5β-cholestan-3α,7α,12α-triacetate (**7a**) exclusively. Thus, the elongation of the C_5 side chain by three carbon atoms to form the C_8 cholestane side chain was accomplished in 5 steps from **2a**; the total yield of **7a** from **2a** was *ca.* 40%.

The C_{27} triacetate **7a**, when treated with methanolic HCl at 30 °C, was deprotected regioselectively at the equatorially-oriented 3α-acetyl group to yield 7α,12α-diacetoxy-5β-cholestan-3α-ol (**8a**), Subsequent oxidation of **8a** with Jones reagent, followed by complete alkaline hydrolysis (using 10% methanolic KOH) of the resulting 3-oxo-7α,12α-diacetoxy derivative (**9a**) proceeded cleanly to give 7α,12α-dihydroxy-5β-cholestan-3-one (**10a**) nearly quantitatively.

The choice of a suitable protecting group for the 7α- and 12α-hydroxy groups in **10a** was an essential factor for subsequent reactions. In our exploratory works, attempted dehydrogenation of **9a** with IBX (see below) was successful, but subsequent hydrolysis of the resulting 7α,12α-diacetoxy-4-cholesten-3-one failed. Under the various alkaline hydrolysis conditions that were examined, allylic elimination of the 7α-acetyl group took place to give a mixture of 12α-hydroxy-4,6-cholestadien-3-one and 12α-acetoxy-4,6-cholestadien-3-one as the major products. To prevent the elimination, we protected the 7α- and 12α-hydroxy groups as formate derivatives. The diformyloxy-5β-cholestan-3-one (**11a**) was found to be preferable to **9a** as a substrate for the IBX reaction, because the formate is more labile and the deprotection is much easy under mild conditions. The compound **11a** was prepared nearly quantitatively from **10a** by the usual formic acid/$HClO_4$ method [10].

Our preliminary experiments revealed that dehydrogenation of **11a** with iodoxybenzene catalyzed by benzeneselenic anhydride was successful [10], but that selective hydrogenation of the Δ^1-bond of the resulting 7α,12α-diformyloxy-1,4-choladien-3-one was unsuccessful. An attempt at direct oxidation of **11a** with selenium dioxide [11] to give 7α,12α-diformyloxy-4-cholesten-3-one (**12a**) also failed.

Recently the report of a new, powerful dehydrogenation oxidant, *o*-iodoxybenzoic acid (IBX), prompted us to apply this reagent for the preparation of the targeted **12a** from **11a**. Nicolaou et al. [12] have reported that IBX, a readily available hypervalent iodine (V) reagent, oxidizes ketones efficiently to the conjugated enones or dienones, and benzylamines to the amines. In addition, IBX was found to be highly effective in mediating dehydrogenations adjacent to carbonyl functionalities (to form α,β-unsaturated carbonyl compounds) as well as at benzylic and related carbon centers (to form conjugated aromatic carbonyl systems). In the IBX-mediated dehydration, some 3-oxo-5α-steroids (*trans* A/B-ring juncture) have been shown to be converted to the corresponding 3-oxo-Δ^1 enones or 3-oxo-$\Delta^{1,4}$ dienones, depending on the experimental conditions. However, Li and Tochtrop [13] have recently reported that IBX smoothly and regioselectively performs the dehydrogenation of 3-oxo-5β-steroids (*cis* A/B-ring juncture) to give the 3-oxo-Δ^4 enones, probably owing to the stereochemical difference of the A/B-rings in the steroid nucleus.

When **11a** was treated with freshly prepared IBX (see Section 3) [14] in DMSO containing trifluoroacetic acid at 40 °C for 40 h, the dehydrogenation reaction took place as expected at the hydrogen atoms attached to C-4 and C-5 to give the desired α,β-unsaturated ketone, 7α,12α-diformyloxy-4-cholesten-3-one (**12a**), in moderate yield (50%), without being accompanied by the elimination of the 7α-formyl group. Also, the undesirable products – 3-oxo-Δ^1-7,12-diformate and 3-oxo-$\Delta^{1,4}$-7,12-diformate – as well as the other by-products, were not formed at all. Based on a suggestion of Nicolaou et al. [12], a possible ionic-mechanism for the dehydrogenation of **11a** by IBX suggests that the reaction is initiated by single electron transfer from the substrate **11a** to IBX to form a radical cation which reacts further to give the desired **12a**.

We found that the use of commercially available stabilized IBX (SIBX), which contains 55 wt.% of 2-iodobenzoic acid as a stabilizer, was unsatisfactory, because it lacked reproducibility, and often resulted in a complicated mixture of products. Isolation of **12a** was tedious and time-consuming, and as a result, was obtained in low yield.

In the final step, the usual alkaline hydrolysis of **12a** with aqueous NaOH at room temperature for 30 min gave the targeted 7α,12α-dihydroxy-4-cholesten-3-one (**1a**). In this hydrolysis reaction of **12a**, the elimination product – 3-oxo-4,6-diene – was produced (2%). On the other hands, when **12b** was treated with aqueous NaOH, 3-oxo-4,6-diene was detected 2% as by-product (yields were established by HPLC). Thus, our successful strategy for obtaining the desired changes in the steroid nucleus was based on solving two problems – first was selection of the appropriate protecting groups for the hydroxy groups of **2a** and **2b** and the second was the use of a modified IBX as the dehydrogenation reagent. Analogous 7α-hydroxy-4-cholesten-3-one (**1b**) (lacking a 12α-hydroxy group) was also prepared starting from CDCA (**2b**) by essentially the same procedures as described in detail for the preparation of **1a** from **2a**. Overall yields of **1a** and **1b** in 11 steps were *ca.* 6.5% and 7.4%, respectively.

2.3. NMR properties

In the ^{1}H-NMR spectrum of **1a**, a pair of doublets appearing at 0.86 and 0.87 ppm were assigned to the three protons at C-26 and C-27 in the terminal isopropyl methyl groups, providing evidence for the structure of the C_8 cholestane side-chain. The 19-H_3 signal (singlet) in **1a** was appreciably shifted downfield by 0.19 ppm and resonated at 1.19 ppm, compared to that in **10a** (1.00 ppm), whereas a signal appearing at 5.82 ppm as a singlet was assigned to the 4-H. These observations indicated that **1a** has the 3-oxo-Δ^4-7α-hydroxy structure in the A/B-ring juncture [15]. Furthermore, the occurrence of the two quaternary ^{13}C signals

at 198.7 and 167.4 ppm and the tertiary ^{13}C signal at 126.9 ppm in the ^{13}C-NMR spectrum of **1a** also indicated the presence of the conjugated enone moiety. The 2D HMBC spectrum of **1a** showed the correlation peaks between 19-H_3 *vs* C-5 and 4-H *vs* C-5, strongly indicating the presence of the Δ^4-bond.

Essentially identical ^1H- and ^{13}C-NMR characteristics were observed in the spectra of **1b**.

2.4. X-ray analysis of 1b

The molecular structure of 7α-hydroxy-4-cholesten-3-one (**1b**) was determined by X-ray analysis. Data for crystallographic details, atomic coordinates, and selected bond lengths (Å) and angles (°) for **1b** are presented in Tables 1–3, respectively. Thus, **1b** crystallizes in a $P2_12_12_1$ space group having four molecules to the unit cell. The molecular structure and the crystal structure of **1b** are presented in Figs. 4 and 5, respectively. The bond distances and bond angles have the expected values for this kind of steroid. In particular, the enone O=C–C=C fragment in ring A is almost anti-periplanar with a small dihedral angle of 3.55° (see Table 3). The bond distances are 1.222(4) for O1-C3, 1.440(4) for C3-C4, and 1.336(4) Å for C4–C5, respectively.

The X-ray crystal structure of **1b** reveals that it is aligned as a zigzag array connected by intermolecular hydrogen bonds between the oxygen atom at C-3 (O1) and the hydroxy-group (O2) of the neighboring molecule along the b axis with an O1···O2 distance of 2.743(3) Å. These arrays are arranged in a head-to-tail manner, and form a molecular layer parallel to the bc-plane. Also shown in Fig. 5, the rigid steroid rings create a large free space into which the side chain is located. Furthermore, because of the absence of strong intermolecular interactions, the C_8 alkyl (cholestane) side chain is quite flexible and, therefore, the thermal motion in that part of the molecule is large. As a consequence, C-26, C-27 and the methine hydrogen atom at the terminal isopropyl group are disordered mutually over three positions due to the free rotation around the C-24–C-25 bond. As a result of the refinements, the site occupancy factor of C-27 was almost 1.0, and C-26 was divided in C-26-1 and C-26-2 with site occupancy factors of 0.60 and 0.40, respectively. The methine hydrogen atom of the isopropyl group was not included in the refinement process. Thus, X-ray analysis provided conclusive evidence for the stereochemical structure of **1b**, resolving the A/B-ring juncture in the steroid nucleus and the terminal isopropyl group at the C_8 cholestane side-chain.

Table 1
Crystallographic details for **1b**.

	1b
Formula	$C_{27}H_{44}O_2$
M	400.64
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	5.893(1)
b (Å)	15.734(3)
c (Å)	26.699(6)
V (Å^3)	2475.6(9)
Z value	4
D_c/g cm^{-3}	1.075
F(000)	888
μ (Mo Kα)/cm^{-1}	0.650
No. reflns measured	29,147
No. unique reflns.	5678
No. observed ($I > 3\sigma$)	3249
R_1 ($I > 3\sigma$)	0.049
wR_2 ($I > 3\sigma$)	0.059
Goodness of fit	0.943

Table 2
Atomic coordinates for **1b**.

Atom	*x*	*y*	*z*	
O1	0.3802(4)	0.59455(13)	0.24964(10)	
O2	0.4221(3)	0.24336(10)	0.28138(7)	
C1	0.1216(5)	0.47800(13)	0.34909(10)	
C2	0.1304(6)	0.55708(15)	0.31535(12)	
C3	0.2649(5)	0.53955(17)	0.26951(13)	
C4	0.2480(5)	0.45526(15)	0.24879(11)	
C5	0.1334(4)	0.39204(15)	0.27063(10)	
C6	0.0990(6)	0.31000(15)	0.24375(10)	
C7	0.1829(5)	0.23469(15)	0.27502(9)	
C8	0.0593(4)	0.23467(13)	0.32505(8)	
C9	0.0939(4)	0.31865(12)	0.35279(8)	
C10	0.0296(4)	0.39988(14)	0.32228(10)	
C11	−0.0210(5)	0.31593(15)	0.40415(10)	
C12	0.0523(5)	0.23932(13)	0.43621(9)	
C13	0.0132(4)	0.15567(13)	0.40896(9)	
C14	0.1386(4)	0.16165(12)	0.35876(8)	
C15	0.1387(6)	0.07132(13)	0.33866(10)	
C16	0.1649(5)	0.01642(15)	0.38624(9)	
C17	0.1299(4)	0.07567(12)	0.43173(8)	
C18	−0.2409(4)	0.13839(17)	0.40204(11)	
C19	−0.2306(4)	0.40816(17)	0.31629(12)	
C20	0.0231(4)	0.03032(13)	0.47663(9)	
C21	−0.0243(6)	0.08980(17)	0.52038(10)	
C22	0.1677(5)	−0.04384(15)	0.49369(9)	
C23	0.0704(5)	−0.09800(16)	0.53488(10)	
C24	0.1985(6)	−0.17933(17)	0.54577(11)	
C25	0.1081(9)	−0.2340(3)	0.58762(14)	
C261	0.1332(15)	−0.1910(4)	0.6351(3)	0.6(occ.)
C262	−0.097(2)	−0.2454(6)	0.5982(4)	0.4(occ.)
C27	0.2296(11)	−0.3163(3)	0.59058(15)	

Table 3
Selected bond lengths (Å) and angles (°) for **1b**.

O1-C3	1.222(4)	O1-C3-C2	121.6(3)
C3-C4	1.440(4)	O1-C3-C4	121.6(3)
C4-C5	1.336(4)	C2-C3-C4	116.8(3)
C5-C6	1.491(4)	C3-C4-C5	123.6(3)
C6-C7	1.531(4)	C3-C4-H6	118.3
C7-C8	1.521(4)	C5-C4-H6	118.1
C7-O2	1.426(4)	C4-C5-C6	120.2(3)
O2-H1	0.840	C5-C6-C7	111.3(3)
		C6-C7-C8	108.9(2)
		C6-C7-O2	108.0(2)
		C7-O2-H1	109.5

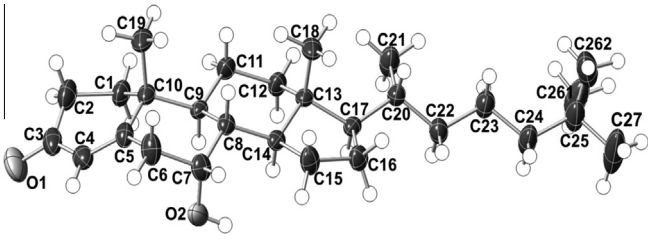


Fig. 4. The molecular structure of **1b** with numbered atoms. Thermal ellipsoids are drawn at 30% of probability.

In conclusion, we report herein the chemical synthesis of **1a** and **1b** from **2a** and **2b**, respectively. The synthesis proceeded through easily prepared, well defined intermediates and provided analytically pure products. The availability of **1a** and **1b** will permit further studies on their plasma levels and facilitate studies on the mechanism by which **1b** (and possibly **1a**) enters plasma, presumably as a component of secreted lipoproteins. It will be of interest to determine whether plasma levels of **1a** also correlate with the rate of bile acid synthesis, and if so, which intermediate (**1a** or

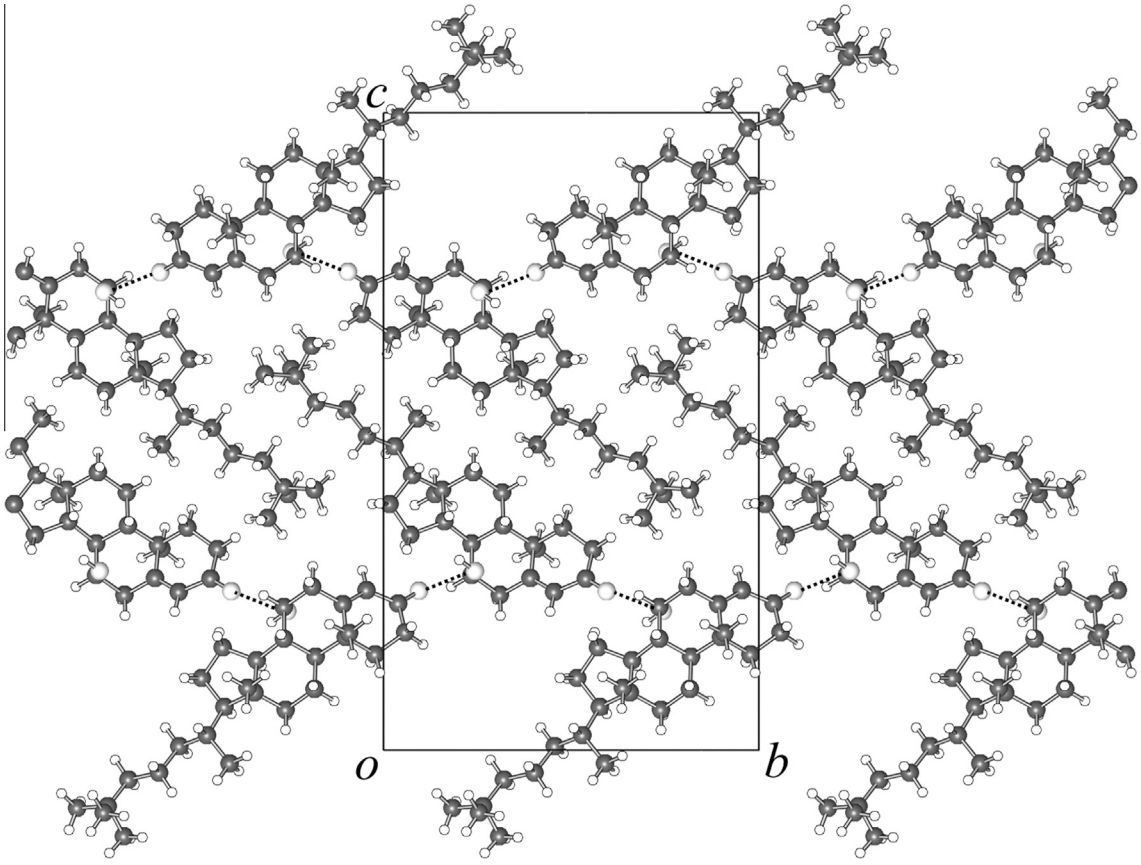


Fig. 5. Crystal structure of **1b** viewed along the *a* axis.

1b) shows a better correlation. In addition, the compounds are now available as substrates for ensuing biosynthetic transformations catalyzed by 12α-hydroxylase (acting on **1b**) as well as the Δ^4-3-oxosteroid 5β-reductase (acting on both **1a** and **1b**) [16].

3. Experimental

3.1. Materials

CA (**2a**) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). CDCA (**2b**) was supplied from Mitsubishi Tanabe Pharma Co. (Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade and available from commercial sources. All compounds were dried by azeotropic distillation prior to use.

3.2. Instruments

All melting points (mp) were determined on a micro hot stage apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL ECA 500 FT instrument operated at 500 and 125.8 MHz, respectively, with $CDCl_3$ or CD_3OD containing 0.1% Me_4Si as the solvent, except where otherwise indicated; chemical shifts were expressed in δ (ppm) relative to Me_4Si. The ^{13}C distortionless enhancement by polarization transfer (DEPT; 135°, 90°, and 45°) spectra were measured to determine the exact ^{13}C signal multiplicity and to differentiate between CH_3, CH_2, CH, and C based on their proton environments. In order to further confirm the ^{1}H and ^{13}C signal assignments for some of compounds, two-dimensional (2D) ^{1}H detected heteronuclear multiple quantum (HMQC; ^{1}H-^{13}C coupling) and ^{1}H detected heteronuclear multiple bond correlation (HMBC; long-range ^{1}H-^{13}C coupling) experiments were also performed.

High-resolution mass spectra by electrospray ionization (HR-ESI-MS) or by atmospheric pressure chemical ionization (HR-APCI-MS) were carried out using a JEOL AccuTOF JMS-T100LC liquid chromatography-mass spectrometer equipped with an ESI source or an APCI source and coupled to a Agilent 1200 series binary pump (Agilent Technologies Inc., Santa Clara, CA, USA) operated in the negative ion mode or positive ion mode.

High-performance liquid chromatography (HPLC) was obtained a Jasco LC-2000 plus HPLC system, consisting of two PU-2085 high-pressure pumps, a MX-2080–32 solvent mixing module, and a CO-2060 column heater equipped with a ChromNAV data processing system (Tokyo, Japan). The column was a Capcell Pack type AQ C18 (particle size, 3 μm, 250 mm × 3.0 mm I.D.; Shiseido, Tokyo, Japan) kept at 37 °C. The detector was an Alltech 2000ES evaporative light-scattering detector (Deerfield, IL, USA) operated under the following conditions: the flow rate of purified compressed air used as a nebulizing gas was 1.8 L/min, and the temperature of the heated drift was 68.3 °C (**1a**) or 65.5 °C (**1b**). The mobile phase used was a mixture of 15 mM-ammonium acetate/acetic acid buffer solution (pH 5.4) and methanol [15:85 (**1a**) or 1:9 (**1b**), v/v]; the flow rate was kept at 0.4 μL/min during the analysis. Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel plates (E. Merck, Darmstad, Germany) using EtOAc–hexane mixtures as the developing solvent.

3.3. Chemical synthesis

3.3.1. 2-Iodoxybenzoic acid (IBX)

IBX was prepared, according to the following procedure [14]. In short, to a solution of 2-iodobenzene (1.0 g, 4 mmol) in water (13 mL) was added OXONE® (monopersulfate compound; $2KHSO_5/KHSO_4/K_2SO_4$; 3.62 g, 5.8 mmol). The resulting suspension was stirred at 70 °C for 3.5 h and then at 5 °C for 1.5 h. The precipitated semi-crystalline solid was filtered, washed with water and acetone, and dried at room temperature for 16 h to afford IBX, which was directly used without further purification. "Caution! IBX is explosive at high temperature."

3.3.2. 3α,7α,12α-Triacetoxy-5β-cholan-24-ol (4a)

A mixture of CA peracetate **3a** (11.8 g, 22.1 mmol), Et_3N (4 mL, 28.7 mmol) in dry THF (100 mL), and ethyl chloroformate (2.8 mL, 29.4 mmol) was stirred at room temperature for 2 h. To the resulting milky turbid suspension, $NaBH_4$ (4.0 g, 106 mmol) and then methanol (10 mL) were added gradually with ice-bath cooling, until a clear solution was obtained. After further stirring at 0 °C for 2 h, the reaction product was extracted with EtOAc. The combined extract was washed with saturated brine, dried over Drierite, and evaporated to dryness. The oily residue was chromatographed on a silica gel column (250 g). Elution with hexane–EtOAc (7:3, v/v) afforded the corresponding 24-hydroxy-3,7,12-triacetate **4a** which was recrystallized from Et_2O–hexane as colorless thin plates; yield, 6.9 g (60%); mp, 134–135 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.74 (s, 3H, 18-H_3), 0.84 (d, 3H, J = 6.3 Hz, 21-H_3), 0.93 (s, 3H, 19-H_3), 2.05, 2.09, 2.14 (each s, 3H, $OCOCH_3$), 3.60 (m, 2H, 24-H_2), 4.56 (m, 1H, 3β-H), 4.91 (brs, 1H, 7β-H), 5.10 (brs, 1H, 12β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.1 (C-18), 17.7 (C-21), 21.3, 21.4, 21.5 (each $OCO\underline{CH_3}$), 22.4 (C-19), 22.7 (C-15), 25.4 (C-11), 26.8 (C-2), 27.1 (C-16), 28.8 (C-9), 29.0 (C-22), 31.1 (C-6), 31.5 (C-23), 34.2 (C-10), 34.5 (C-4), 34.6 (C-1), 34.7 (C-20), 37.6 (C-8), 40.8 (C-5), 43.3 (C-14), 44.9 (C-13), 47.4 (C-17), 63.2 (C-24), 70.6 (C-7), 74.0 (C-3), 75.4 (C-12), 170.3, 170.5, 170.5 (each $O\underline{CO}CH_3$). HR-ESI-MS, calcd. for $C_{30}H_{49}O_7$ $[M+H]^+$, 521.3478; found 521.3431.

3.3.3. 3α,7α-Diacetoxy-5β-cholan-24-ol (4b)

The C-24 carboxyl group of CDCA diacetate **3b** (12.0 g, 25 mmol) [obtained from CDCA (**2b**)], was reduced by the procedure described for the preparation of **4a** to give 24-hydroxy-3,7-diacetate **4b**, which was recrystallized from Et_2O-hexane as colorless thin plates; yield, 6.9 g (59%); mp, 160–162 °C (literature value: mp, 159 °C) [17]. ^{1}H-NMR (500 MHz, $CDCl_3$): = δ 0.65 (s, 3H, 18-H_3), 0.93 (s, 3H, 19-H_3), 0.94 (d, 3H, J = 5.2 Hz, 21-H_3), 2.05, 2.09 (each s, 3H, $OCOCH_3$), 3.61 (m, 2H, 24-H_2), 4.59 (m, 1H, 3β-H), 4.88 (brs, 1H, 7β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 11.7 (C-18), 18.5 (C-21), 20.6 (C-11), 21.4, 21.6 (each $OCO\underline{CH_3}$), 22.6 (C-19), 23.5 (C-15), 26.7 (C-2), 28.1 (C-16), 29.2 (C-23), 31.4 (C-6), 31.7 (C-22), 34.0 (C-10), 34.7 (C-8), 34.8 (C-4), 34.9 (C-1), 35.4 (C-20), 37.9 (C-9), 39.5 (C-12), 40.9 (C-5), 42.6 (C-13), 50.4 (C-14), 55.9 (C-17), 63.5 (C-24), 71.2 (C-7), 74.2 (C-3), 170.5, 170.6 ($O\underline{CO}CH_3$). HR-APCI-MS, calcd. for $C_{28}H_{47}O_5$ $[M+H]^+$, 463.3424; found 463.3439.

3.3.4. 3α,7α,12α-Triacetoxy-5β-cholan-24-al (5a)

To a solution of the 24-hydroxy-3,7,12-triacetate **4a** (1.0 g, 1.9 mmol) in CH_2Cl_2 (50 mL), 2,2,6,6-tetramethylpiperidine1-oxyl free radical (TEMPO) (7 mg, 45 μmol), and KBr (24 mg) were added gradually with ice-bath cooling. To the mixture, a solution of NaOCl (2 mL) dissolved in a buffer solution (pH 8.6, 14 mL) prepared from 0.5 M sodium bicarbonate, and 0.05 M sodium carbonate was added. The mixture was stirred at 0 °C for 20 min; the reaction was monitored by TLC using hexane–EtOAc (1:1, v/v) as the developing solvent. After adding methanol (1 mL), the reaction product was extracted with CH_2Cl_2. The combined extract was washed with water, dried over Drierite, and evaporated to dryness to give the crude 24-aldehyde-3,7,12-triacetate **5a**. Recrystallization from methanol afforded the analytically pure **5a** as colorless thin plates; yield, 790 mg (80%); mp, 86–87 °C (literature value: mp, 55 °C) [18]. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.73 (s, 3H, 18-H_3), 0.82 (d, 3H, J = 5.4 Hz, 21-H_3), 0.92 (s, 3H, 19-H_3), 2.05, 2.09, 2.14 (each s, 3H, $OCOCH_3$), 4.58 (m, 1H, 3β-H), 4.91 (brs, 1H, 7β-H), 5.09 (brs, 1H, 12β-H), 9.76 (s, 1H, 24-CHO). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.2 (C-18), 17.6 (C-21), 21.4, 21.5, 21.6 (each $OCO\underline{CH_3}$), 22.5 (C-19), 22.9 (C-15), 25.7 (C-11), 27.0 (C-2), 27.3 (C-16), 27.7 (C-22), 29.0 (C-9), 31.3 (C-6), 34.2 (C-10), 34.5 (C-4), 34.7 (C-1), 34.4 (C-20), 37.9 (C-8), 40.9 (C-23), 41.0 (C-5), 43.5 (C-14), 45.2 (C-13), 47.5 (C-17), 70.7 (C-7), 74.1 (C-3), 75.3 (C-12), 170.3, 170.5, 170.5 (each $O\underline{CO}CH_3$), 202.8 (C-24). HR-ESI-MS, calcd. for $C_{30}H_{47}O_7$ $[M+H]^+$, 519.3322; found 519.3315.

3.3.5. 3α,7α-Diacetoxy-5β-cholan-24-al (5b)

The 24-hydroxy-3,7-diacetate **4b** (1.0 g, 2.16 mmol) was converted to the corresponding 24-aldehyde-3,7-diacetate **5b** by the TEMPO oxidation as described for the preparation of **5a**. The crude **5b** was recrystallized from Et_2O as colorless thin plates; yield, 882 mg (89%); mp, 153–155 °C (literature value: mp, 153–154 °C) [17]. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.63 (s, 3H, 18-H_3), 0.92 (d, 3H, J = 6.3 Hz, 21-H_3), 0.93 (s, 3H, 19-H_3), 2.03, 2.06 (each s, 3H, $OCOCH_3$), 4.59 (m, 1H, 3β-H), 4.88 (brs, 1H, 7β-H), 9.76 (s, 1H, 24-CHO). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 11.7 (C-18), 18.4 (C-21), 20.6 (C-11), 21.5, 21.6 (each $OCO\underline{CH_3}$), 22.7 (C-19), 23.5 (C-15), 26.8 (C-2), 28.0 (C-16), 28.1 (C-22), 31.3 (C-6), 34.1 (C-20), 34.6 (C-10), 34.8 (C-4), 34.9 (C-1), 35.3 (C-9), 37.9 (C-8), 39.5 (C-12), 40.9 (C-23), 41.0 (C-5), 42.7 (C-13), 50.4 (C-14), 55.8 (C-17), 71.2 (C-7), 74.1 (C-3), 170.4, 170.6 (each $O\underline{CO}CH_3$), 203.1 (C-24). HR-APCI-MS, calcd. for $C_{28}H_{45}O_5$ $[M+H]^+$, 461.3267; found 461.3221.

3.3.6. 3α,7α,12α-Triacetoxy-5β-cholest-24-ene (6a)

To a magnetically stirred solution of isopropyltriphenylphosphonium iodide (230 mg, 0.53 mmol) in dry THF (5 mL), a solution of 1.6 M n-BuLi in hexane (330 μL) was added slowly with ice-bath cooling. The mixture was stirred at 0 °C for 30 min. To this mixture, a solution of the 24-aldehyde-3,7,12-triacetate **5a** (100 mg, 0.19 mmol) and tri[2-(2-methoxyethoxy)ethyl]amine (10 μL) dissolved in dry THF (5 mL) was then added, and the mixture was further stirred at 0 °C for 15 min. After quenching the reaction by the addition of acetone (2 mL), the reaction product was extracted with CH_2Cl_2. The combined extract was washed with water, dried over Drierite, and evaporated to give a light yellow residue. Acetylation of the residue by the usual method and subsequent work-up by solvent extraction gave the crude reaction product. Chromatography of the product on a column of silica gel (5 g) and elution with hexane–EtOAc (4:1, v/v) afforded the desired Δ^{24}-3,7,12-triacetate **6a** which recrystallized from methanol as colorless needles; yield, 63 mg (60%); mp, 84–86 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.73 (s, 3H, 18-H_3), 0.82 (d, 3H, J = 8.1 Hz, 21-H_3), 0.92 (s, 3H, 19-H_3), 1.59 (s, 3H, 26-H_3), 1.68 (s, 3H, 27-H_3), 2.05, 2.09, 2.14 (each s, 3H, $OCOCH_3$), 4.58 (m, 1H, 3β-H), 4.92 (brs, 1H, 7β-H), 5.07 (t, 1H, J = 3.4 Hz, 24-H), 5.10 (brs, 1H, 12β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.2 (C-18), 17.6 (C-26), 17.8 (C-21), 21.4, 21.4, 21.6 (each $OCO\underline{CH_3}$), 22.5 (C-19), 22.8 (C-15), 24.6 (C-23), 25.5 (C-11), 25.7 (C-27), 26.8 (C-2), 27.2 (C-16), 28.8 (C-9), 31.2 (C-6), 34.3 (C-10), 34.6 (C-4), 34.7 (C-1), 34.8 (C-20), 35.8 (C-22), 37.7 (C-8), 40.9 (C-5), 43.3 (C-14), 45.0 (C-13), 47.7 (C-17), 70.7 (C-7), 74.1 (C-3), 75.4 (C-12), 124.8 (C-24), 131.1 (C-25), 170.3, 170.5, 170.5 (each $O\underline{CO}CH_3$). HR-APCI-MS, calcd. for $C_{33}H_{53}O_6$ $[M+H]^+$, 545.3842; found 545.3824.

3.3.7. 3α,7α-Diacetoxy-5β-cholest-24-ene (6b)

The 24-aldehyde-3,7-diacete **5b** (900 mg 1.95 mmol), was subjected to the Wittig reaction with isopropyltriphenylphosphonium iodide and processed as described for the preparation of **6a**. The procedure afforded the Δ^{24}-3,7-diacetate **6b** which crystallized from methanol as colorless needles; yield, 552 mg (58%); mp, 74–76 °C (literature value: mp, 67–69 °C) [19]. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.64 (s, 3H, 18-H_3), 0.92 (d, 3H, J = 5.0 Hz, 21-H_3), 0.93 (s, 3H, 19-H_3), 1.60 (s, 3H, 27-H_3), 1.68 (s, 3H, 26-H_3), 2.03, 2.05 (each s, 3H, $OCOCH_3$), 4.59 (m, 1H, 3β-H), 4.88 (brs, 1H, 7β-H), 5.09 (m, 1H, 24-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 11.6 (C-18), 17.6 (C-26), 18.5 (C-21), 20.5 (C-11), 21.4, 21.5 (each $OCOCH_3$), 22.6 (C-19), 23.5 (C-15), 24.7 (C-23), 25.7 (C-27), 26.7 (C-2), 28.2 (C-16), 31.3 (C-6), 34.0 (C-9), 34.6 (C-4), 34.8 (C-10), 34.9 (C-1), 35.6 (C-20), 36.1 (C-22), 37.9 (C-8), 39.5 (C-12), 40.9 (C-5), 42.7 (C-13), 50.4 (C-14), 56.1 (C-17), 71.3 (C-7), 74.2 (C-3), 125.1 (C-24), 131.0 (C-25), 170.5, 170.6 (each $OCOCH_3$). HR-ESI-MS, calcd. for $C_{31}H_{51}O_4$ $[M+H]^+$, 487.3787; found 487.3753.

3.3.8. 3α,7α,12α-Triacetoxy-5β-cholestane (7a)

The Δ^{24}-3,7,12-triacetate **6a** (60 mg, 0.11 mmol) in a mixed solvent (20 mL) of EtOAc-methanol-acetic acid (10:10:1, v/v/v) was hydrogenated in the presence of 10% Pd/C catalyst (6 mg) under a slight positive pressure. After stirring at room temperature for 3 h, the catalyst was removed by filtration through Celite, and the solvent of the mother liquor was evaporated under reduced pressure to give a pale yellow oil of 5β-cholestane 3,7,12-triacetate (**7a**). Although **7a** was found to be homogeneous according to TLC, HPLC, and NMR analyses, it resisted crystallization attempts; yield, 58.8 mg (98%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.72 (s, 3H, 18-H_3), 0.79 (d, 3H, J = 6.8 Hz, 21-H_3), 0.84 (d, 6H, J = 6.8 Hz, 26-H_3 and 27-H_3), 0.91 (s, 3H, 19-H_3), 2.03, 2.07, 2.12 (each s, 3H, $OCOCH_3$), 4.56 (m, 1H, 3β-H), 4.89 (brs, 1H, 7β-H), 5.09 (brs, 1H, 12β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.0 (C-18), 17.7 (C-21), 21.2, 21.3, 21.4 (each $OCOCH_3$), 22.4 (C-19 and C-26), 22.6 (C-27), 22.7 (C-23), 23.7 (C-15), 25.4 (C-11), 26.9 (C-2), 27.1 (C-16), 27.8 (C-25), 28.7 (C-9), 31.1 (C-6), 34.2 (C-10), 34.5 (C-1 and C-4), 34.9 (C-20), 35.8 (C-22), 37.6 (C-8), 39.2 (C-24), 40.8 (C-5), 43.2 (C-14), 44.8 (C-13), 47.6 (C-17), 70.6 (C-7), 73.9 (C-3), 75.3 (C-12), 170.2, 170.3, 170.3 (each $OCOCH_3$). HR-APCI-MS, cald. for $C_{33}H_{55}O_6$ $[M+H]^+$, 547.3999; found 547.3988.

3.3.9. 3α,7α-Diacetoxy-5β-cholestane (7b)

Catalytic hydrogenation of Δ^{24}-3,7-diacetate **6b** (100 mg, 0.21 mmol) by the procedure as described for the preparation of **7a** gave the 5β-cholestane 3,7-diacetate (**7b**); yield, 98 mg (98%); mp, 85–87 °C (recrystallized from methanol as colorless thin plates) (literature value: mp, 83–87 °C) [20]. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.64 (s, 3H, 18-H_3), 0.87 (d, 6H, J = 6.8 Hz, 26-H_3 and 27-H_3), 0.92 (d, 3H, J = 5.0 Hz, 21-H_3), 0.93 (s, 3H, 19-H_3), 2.04, 2.06 (each s, 3H, $OCOCH_3$), 4.59 (m, 1H, 3β-H), 4.88 (brs, 1H, 7β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 11.7 (C-18), 18.6 (C-21), 20.6 (C-11), 21.5, 21.6 (each $OCOCH_3$), 22.5 (C-26), 22.7 (C-19), 22.8 (C-27), 23.6 (C-23), 23.8 (C-15), 26.8 (C-2), 28.0 (C-25), 28.1 (C-16), 31.3 (C-6), 34.0 (C-9), 34.6 (C-4), 34.8 (C-10), 34.9 (C-1), 35.7 (C-20), 36.1 (C-22), 37.9 (C-8), 39.4 (C-12), 39.5 (C-24), 40.9 (C-5), 42.6 (C-13), 50.4 (C-14), 56.2 (C-17), 71.3 (C-7), 74.2 (C-3), 170.4, 170.6 (each $OCOCH_3$). HR-APCI-MS calcd. for $C_{31}H_{53}O_4$ $[M+H]^+$, 489.3944; found 489.3938.

3.3.10. 7α,12α-Diacetoxy-5β-cholestan-3α-ol (8a)

A mixture of the 5β-cholestane 3,7,12-triacetate **7a** (500 mg, 0.91 mmol) and conc. HCl (6 drops) in methanol (15 mL) was slow stirred at 30 °C for 6 h. Most of the solvent was evaporated under reduced pressure, and the reaction product was extracted with EtOAc. The combined extract was washed with saturated brine, dried over Drierite, and evaporated to dryness. The oily residue was chromatographed on a column of silica gel (15 g). Elution with EtOAc-hexane (6:4, v/v) gave the 3-hydroxy-7,12-diacetate **8a**, which was crystallized from methanol as colorless needles; yield, 334 mg (72%); mp, 138–141 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.72 (s, 3H, 18-H_3), 0.79 (d, 3H, J = 6.3 Hz, 21-H_3), 0.86 (d, 6H, J = 6.8 Hz, 26-H_3 and 27-H_3), 0.91 (s, 3H, 19-H_3), 2.09, 2.13 (each s, 3H, $OCOCH_3$), 3.50 (m, 1H, 3β-H), 4.90 (brs, 1H, 7β-H), 5.10 (brs, 1H, 12β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.2 (C-18), 17.8 (C-21), 21.4, 21.6 (each $OCOCH_3$), 22.5 (C-19 and C-26), 22.8 (C-23 and C-27), 23.8 (C-15), 25.5 (C-11), 27.3 (C-16), 28.0 (C-25), 28.9 (C-9), 30.4 (C-2), 31.3 (C-6), 34.3 (C-10), 34.8 (C-1), 35.1 (C-20), 35.9 (C-22), 37.7 (C-8), 38.6 (C-4), 39.4 (C-24), 41.0 (C-5), 43.3 (C-14), 45.0 (C-13), 47.7 (C-17), 70.9 (C-7), 71.7 (C-3), 75.6 (C-12), 170.6, 170.7 (each $OCOCH_3$). HR-APCI-MS calcd. for $C_{31}H_{51}O_5$ $[M-H]^-$, 503.3737; found, 503.3741.

3.3.11. 7α-Acetoxy-5β-cholestan-3α-ol (8b)

3-Hydroxy-7-acetate **8b** was prepared from the 5β-cholestane 3,7-diacetate **7b** (600 mg, 1.23 mmol) by selective hydrolysis at C-3 using concentrated HCl as described for the preparation of **8a**. Crystallization of the crude product from EtOAc gave colorless needles; yield, 437 mg (80%); mp, 145–146 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.64 (s, 3H, 18-H_3), 0.87 (d, 6H, J = 6.8 Hz, 26-H_3 and 27-H_3), 0.92 (d, 3H, J = 5.0 Hz, 21-H_3), 0.93 (s, 3H, 19-H_3), 2.05 (s, 3H, $OCOCH_3$), 3.50 (m, 1H, 3β-H), 4.88 (brs, 1H, 7β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 11.6 (C-18), 18.6 (C-21), 20.6 (C-11), 21.6 ($OCOCH_3$), 22.5 (C-26), 22.7 (C-19), 22.8 (C-27), 23.6 (C-23), 23.8 (C-15), 28.0 (C-16), 28.1 (C-25), 30.6 (C-2), 31.4 (C-6), 34.1 (C-9), 34.7 (C-10), 35.2 (C-1), 35.7 (C-20), 36.1 (C-22), 37.9 (C-8), 38.9 (C-4), 39.4 (C-12), 39.5 (C-24), 41.1 (C-5), 42.6 (C-13), 50.4 (C-14), 56.1 (C-17), 71.4 (C-7), 71.8 (C-3), 170.7 ($OCOCH_3$). HR-ESI-MS, calcd. for $C_{29}H_{49}O_3$ $[M-H]^-$, 445.3682; found, 445.3647.

3.3.12. 7α,12α-Diacetoxy-5β-cholestan-3-one (9a)

Jones reagent (0.6 mL) was added gradually to a stirred solution of the 3-hydroxy-7,12-diacetate **8a** (300 mg, 0.59 mmol) in acetone (8 mL) and CH_2Cl_2 (1.5 mL) until the solution became dark brown. After stirring at 0 °C for 30 min, 2-propanol (0.8 mL) was added to the mixture. The reaction product was extracted with CH_2Cl_2. The combined extract was washed with water, dried over Drierite, and evaporated to afford the 3-oxo-7,12-diacetate **9a** which crystallized from EtOAc as colorless needles; yield, 248 mg (83%); mp, 171–172 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.77 (s, 3H, 18-H_3), 0.82 (d, 3H, J = 5.4 Hz, 21-H_3), 0.86 (d, 6H, J = 5.4 Hz, 26-H_3 and 27-H_3), 1.02 (s, 3H, 19-H_3), 2.07, 2.12 (each s, 3H, $OCOCH_3$), 5.00 (brs, 1H, 7β-H), 5.15 (brs, 1H, 12β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.2 (C-18), 17.8 (C-21), 21.3, 21.5 (each $OCOCH_3$), 21.6 (C-19), 22.5 (C-26), 22.8 (C-27), 23.8 (C-15 and C-23), 25.8 (C-11), 27.2 (C-16), 27.9 (C-25), 29.8 (C-9), 30.9 (C-6), 34.4 (C-10), 35.0 (C-20), 35.9 (C-22), 36.1 (C-1), 36.6 (C-2), 37.7 (C-8), 39.3 (C-24), 42.4 (C-5), 43.2 (C-14), 44.6 (C-4), 45.0 (C-13), 47.8 (C-17), 70.6 (C-7), 75.3 (C-12), 170.4, 170.5 (each $OCOCH_3$), 212.4 (C-3). HR-APCI-MS, calcd. for $C_{31}H_{51}O_5$ $[M+H]^+$, 503.3737; found, 503.3701.

3.3.13. 7α-Acetoxy-5β-cholestan-3-one (9b)

The 3-hydroxy-7-acetate **8b** (400 mg, 0.90 mmol), subjected to the oxidation reaction with Jones reagent and processed as described for the preparation of **9a**, afforded a homogeneous oily product of the 3-oxo-7-acetate **9b**, which resisted crystallization attempts; yield, 372 mg (93%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.68 (s, 3H, 18-H_3), 0.87 (d, 6H, J = 6.8 Hz, 26-H_3 and 27-H_3), 0.92 (d, 3H, J = 5.0 Hz, 21-H_3), 1.04 (s, 3H, 19-H_3), 2.04 (s, 3H, $OCOCH_3$), 4.96 (brs, 1H, 7β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$):

δ = 11.7 (C-18), 18.7 (C-21), 21.0 (C-11), 21.5 (OCO<u>CH$_3$</u>), 21.8 (C-19), 22.5 (C-26), 22.8 (C-27), 23.6 (C-23), 23.8 (C-15), 28.0 (C-16), 28.1 (C-25), 31.0 (C-6), 34.9 (C-9) 35.0 (C-10), 35.7 (C-20), 36.1 (C-22), 36.6 (C-2), 36.9 (C-1), 37.9 (C-8), 39.4 (C-12 and C-24), 42.6 (C-5), 42.7 (C-13), 44.7 (C-4), 50.3 (C-14), 56.1 (C-17), 71.2 (C-7), 170.3 (O<u>CO</u>CH$_3$), 212.6 (C-3). HR-APCI-MS, calcd. for $C_{29}H_{49}O_3$ $[M + H]^+$, 445.3682; found, 445.3680.

3.3.14. 7α,12α-Dihydroxy-5β-cholestan-3-one (10a)

The 3-oxo-7,12-diacetate **9a** (200 mg, 0.40 mmol) was completely hydrolyzed in 10% methanolic KOH (3.5 g) at 30 °C for 16 h. Most of the solvent was evaporated off, and the residue was dissolved in water and acidified with 10% HCl with stirring and ice-bath cooling. The precipitate was collected by filtration and washed with water. Recrystallization from methanol gave the 7,12-dihydroxy-3-one **10a** as colorless thin plates; yield, 149 mg (90%); mp, 195–197 °C (literature value: mp, 209–210 °C) [21]. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.74 (s, 3H, 18-H$_3$), 0.86 (d, 3H, J = 6.0 Hz, 27-H$_3$), 0.87 (d, 3H, J = 6.0 Hz, 26-H$_3$), 0.98 (d, 3H, J = 6.5 Hz, 21-H$_3$), 1.00 (s, 3H, 19-H$_3$), 3.93 (brs, 1H, 7β-H), 4.06 (brs, 1H, 12β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.6 (C-18), 17.8 (C-21), 21.7 (C-19), 22.5 (C-26), 22.8 (C-27), 23.1 (C-23), 23.8 (C-15), 27.3 (C-9), 27.5 (C-16), 28.0 (C-25), 28.5 (C-11), 33.7 (C-6), 34.9 (C-10), 35.4 (C-20), 36.0 (C-22), 36.6 (C-1), 36.8 (C-2), 39.4 (C-24), 39.6 (C-8), 41.9 (C-14), 43.1 (C-5), 45.6 (C-4), 46.6 (C-13), 47.7 (C-17), 68.3 (C-7), 72.9 (C-12), 213.1 (C-3). HR-APCI-MS, calcd. for $C_{27}H_{45}O_3$ $[M-H]^-$, 417.3369; found, 417.3361.

3.3.15. 7α-Hydroxy-5β-cholestan-3-one (10b)

The 3-oxo-7-acetate **9b** (300 mg, 0.67 mmol), subjected to complete hydrolysis with 10% methanolic KOH (6 g) at 30 °C for 16 h and processed as described above, afforded the 7-hydroxy-3-one **10b**; yield, 266 mg (98%); mp, 62–65 °C (colorless needles from petroleum ether) (literature value: mp, 122–123 °C) [22]. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.70 (s, 3H, 18-H$_3$), 0.87 (d, 6H, J = 5.4 Hz, 26-H$_3$ and 27-H$_3$), 0.93 (d, 3H, J = 5.4 Hz, 21-H$_3$), 1.01 (s, 3H, 19-H$_3$), 3.93 (brs, 1H, 7β-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 11.8 (C-18), 18.7 (C-21), 21.0 (C-11), 21.9 (C-19), 22.6 (C-26), 22.8 (C-27), 23.7 (C-23), 23.8 (C-15), 28.0 (C-25), 28.2 (C-16), 33.4 (C-9), 33.8 (C-6), 35.3 (C-10), 35.8 (C-20), 36.2 (C-22), 36.8 (C-1), 37.0 (C-2), 39.4 (C-8), 39.5 (C-12), 39.6 (C-24), 42.7 (C-13), 43.2 (C-5), 45.6 (C-4), 50.4 (C-14), 56.2 (C-17), 68.6 (C-7), 213.2 (C-3). HR-ESI-MS, calcd. for $C_{27}H_{45}O_2$ $[M-H]^-$, 401.3420; found 401.3434.

3.3.16. 7α,12α-Diformyloxy-5β-cholestan-3-one (11a)

A solution of the 7,12-dihydroxy-3-one **10a** (200 mg, 0.48 mmol) in 98% formic acid (2 mL) containing one drop of 60% perchloric acid was stirred at room temperature for 1 h. Acetic anhydride (2 mL) was added slowly with ice-bath cooling, and then the mixture was poured into ice. The precipitated solid was filtered, washed with water, and the product (**11a**) was recrystallized from methanol **11a** as colorless thin plates; yield, 218 mg (96%); mp, 176–178 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.79 (s, 3H, 18-H$_3$), 0.86 (d, 9H, J = 6.5 Hz, 21-H$_3$ and 26-H$_3$ and 27-H$_3$), 1.04 (s, 3H, 19-H$_3$), 5.16 (brs, 1H, 7β-H), 5.32 (brs, 1H, 12β-H), 8.10, 8.16 (each s, 1H, OCHO). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.2 (C-18), 17.8 (C-21), 21.6 (C-19), 22.5 (C-26), 22.8 (C-15 and C-27), 23.7 (C-23), 25.9 (C-11), 27.2 (C-16), 27.9 (C-25), 29.5 (C-9), 31.0 (C-6), 34.4 (C-10), 35.2 (C-20), 35.8 (C-22), 36.1 (C-1), 36.5 (C-2), 37.7 (C-8), 39.4 (C-24), 42.2 (C-5), 42.8 (C-14), 44.6 (C-4), 45.0 (C-13), 47.5 (C-17), 70.6 (C-7), 75.3 (C-12), 160.3, 160.5 (each OCHO), 211.7 (C-3). HR-APCI-MS, calcd for $C_{29}H_{47}O_5$ $[M + H]^+$, 475.3424; found 475.3458.

3.3.17. 7α-Formyloxy-5β-cholestan-3-one (11b)

The 7-hydroxy-3-one **10b** (250 mg, 0.62 mmol) was converted to the 3-oxo-7-formate **11b** by the method described for the preparation of **11a**. Although this compound was analytically pure by TLC, and NMR, it resisted crystallization attempts; yield, 182 mg (68%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.69 (s, 3H, 18-H$_3$), 0.86 (d, 3H, J = 6.0 Hz, 27-H$_3$), 0.87 (d, 3H, J = 6.0 Hz, 26-H$_3$), 0.92 (d, 3H, J = 6.6 Hz, 21-H$_3$), 1.04 (s, 3H, 19-H$_3$), 5.12 (brs, 1H, 7β-H) 8.06 (s, 1H, OCHO). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 11.7 (C-18), 18.7 (C-21), 21.0 (C-11), 21.8 (C-19), 22.5 (C-26), 22.8 (C-27), 23.5 (C-23), 23.7 (C-15), 28.0 (C-25), 28.1 (C-16), 31.2 (C-6), 34.8 (C-9), 35.0 (C-10), 35.7 (C-20), 36.1 (C-22), 36.5 (C-1), 36.8 (C-2), 37.9 (C-8), 39.4 (C-12 and C-24), 42.6 (C-5), 42.7 (C-13), 44.7 (C-4), 50.0 (C-14), 56.0 (C-17), 71.3 (C-7), 160.5 (OCHO), 212.1 (C-3). HR-APCI-MS, calcd. for $C_{28}H_{47}O_3$ $[M + H]^+$, 431.3525; found 431.3545.

3.3.18. 7α,12α-Diformyloxy-4-cholesten-3-one (12a)

To a magnetically stirred solution of the 3-oxo-7,12-diformate **11a** (50 mg, 0.11 mmol) in DMSO (5 mL) was added freshly prepared IBX (40 mg, 0.28 mmol) and two drops of trifluoroacetic acid; the mixture was stirred at 40 °C for 40 h. After cooling to room temperature, the reaction product was extracted with EtOAc, and the combined extract was washed with saturated brine, dried over Drierite, and evaporated to dryness. The crude product was purified on a silica gel column (3 g). Elution with EtOAc-hexane (1:4, v/v) yielded the conjugated 3-oxo-Δ^4-7,12-diformate **12a** which was recrystallized from methanol as colorless prisms; yield, 28 mg (56%); mp, 134–136 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.81 (s, 3H, 18-H$_3$), 0.86 (d, 9H, J = 6.5 Hz, 21-H$_3$ and 26-H$_3$ and 27-H$_3$), 1.21 (s, 3H, 19-H$_3$), 5.19 (brs, 1H, 7β-H), 5.30 (brs, 1H, 12β-H), 5.71 (s, 1H, 4-H), 8.08, 8.11 (each s, 1H, OCHO). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.1 (C-18), 16.8 (C-19), 17.8 (C-21), 22.5 (C-26), 22.8 (C-15 and C-27), 23.7 (C-23), 25.6 (C-11), 27.1 (C-16), 27.9 (C-25), 33.7 (C-2), 35.1 (C-1), 35.2 (C-20), 35.8 (C-22), 37.3 (C-6), 37.6 (C-10), 38.2 (C-9), 39.4 (C-24), 40.6 (C-8), 42.8 (C-14), 44.8 (C-13), 47.4 (C-17), 70.6 (C-7), 74.8 (C-12), 126.8 (C-4), 160.3, 160.4 (each OCHO), 165.0 (C-5), 198.4 (C-3). HR-APCI-MS, calcd. for $C_{29}H_{45}O_5$ $[M + H]^+$, 473.3267; found 473.3307.

3.3.19. 7α-Formyloxy-4-cholesten-3-one (12b)

The 3-oxo-7-formate **11b** (200 mg, 0.46 mmol), subjected to the dehydration with IBX and processed as described for the preparation of **12a**, afforded an oily residue. Chromatography of the oily product on a silica gel column (10 g) and elution with EtOAc-hexane (1:9, v/v) gave the conjugated 3-oxo-Δ^4-7-formate **12b** which recrystallized from acetone as colorless thin plates; yield, 127 mg (64%); mp, 152–155 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.71 (s, 3H, 18-H$_3$), 0.87 (d, 6H, J = 6.8 Hz, 26-H$_3$ and 27-H$_3$), 0.92 (d, 3H, J = 5.0 Hz, 21-H$_3$), 1.22 (s, 3H, 19-H$_3$), 5.17 (brs, 1H, 7β-H) 5.70 (s, 1H, 4-H), 8.05 (s, 1H, OCHO). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 11.7 (C-18), 17.1 (C-19), 18.6 (C-21), 20.8 (C-11), 22.5 (C-26), 22.8 (C-27), 23.5 (C-23), 23.7 (C-15), 28.0 (C-16 and C-25), 33.9 (C-2), 35.4 (C-1), 35.6 (C-20), 36.0 (C-22), 37.4 (C-6), 38.2 (C-10), 38.3 (C-8), 39.0 (C-12), 39.4 (C-24), 42.4 (C-13), 46.2 (C-9), 50.0 (C-14), 55.9 (C-17), 71.2 (C-7), 126.5 (C-4), 160.4 (OCHO), 166.1 (C-5), 198.8 (C-3). HR-APCI-MS, calcd. for $C_{28}H_{45}O_3$ $[M + H]^+$, 429.3369; found 429.3411.

3.3.20. 7α,12α-Dihydroxy-4-cholesten-3-one (1a)

A solution of the 3-oxo-Δ^4-7,12-diformate **12a** (15 mg, 32 μmol) in THF (1 mL) and 0.8% aqueous NaOH (1 mL) was left to stand at room temperature for 30 min; the reaction was monitored by TLC. The solution was diluted with water, acidified by 10% HCl with ice-bath cooling, and the precipitated solid was collected by filtration, washed with water. Recrystallization from

methanol gave the desired 3-oxo-Δ^4-7,12-dihydroxy compound **1a** as colorless needles; yield, 11 mg (80%); mp, 147–148 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.75 (s, 3H, 18-H_3), 0.86 (d, 3H, J = 6.8 Hz, 27-H_3), 0.87 (d, 3H, J = 6.8 Hz, 26-H_3), 0.98 (d, 3H, J = 6.0 Hz, 21-H_3), 1.19 (s, 3H, 19-H_3), 3.98 (brs, 1H, 7β-H), 4.05 (brs, 1H, 12β-H), 5.82 (s, 1H, 4-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 12.6 (C-18), 16.9 (C-19), 17.8 (C-21), 22.5 (C-26), 22.8 (C-27), 23.0 (C-15), 23.8 (C-23), 27.4 (C-16), 28.0 (C-25), 28.3 (C-11), 33.9 (C-2), 35.2 (C-1), 35.4 (C-20), 36.0 (C-22), 38.0 (C-10), 38.9 (C-9), 39.5 (C-24), 40.0 (C-8), 40.8 (C-6), 42.0 (C-14), 46.4 (C-13), 47.6 (C-17), 68.3 (C-7), 72.5 (C-12), 126.9 (C-4), 167.4 (C-5), 198.7 (C-3). HR-ESI-MS, calcd. for $C_{27}H_{43}O_3$ $[M-H]^-$, 415.3212; found 415.3211.

3.3.21. 7α-Hydroxy-4-cholesten-3-one (1b)

The 3-oxo-Δ^4-diformate **12b** (100 mg, 0.23 mmol) in THF (5 mL) was hydrolyzed with 0.8% aqueous NaOH, followed by acidification with 10% HCl as described for the preparation of **1a**. After being processed analogously, the crude product dissolved in benzene was purified using a column of silica gel (5 g), and eluting with EtOAc-hexane (3:7, v/v). Recrystallization from methanol gave the 3-oxo-Δ^4-7-hydroxy compound **1b** as colorless needles; yield, 73 mg (78%); mp, 180–182 °C (literature value: mp, 183–184 °C) [23]. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.71 (s, 3H, 18-H_3), 0.89 (d, 3H, J = 6.8 Hz, 27-H_3), 0.90 (d, 3H, J = 6.8 Hz, 26-H_3), 0.91 (d, 3H, J = 5.4 Hz, 21-H_3), 1.20 (s, 3H, 19-CH_3), 3.97 (brs, 1H, 7β-H), 5.80 (s, 1H, 4-H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 11.8 (C-18), 17.0 (C-19), 18.6 (C-21), 20.8 (C-11), 22.5 (C-26), 22.8 (C-27), 23.5 (C-23), 23.7 (C-15), 28.0 (C-25), 28.1 (C-16), 33.9 (C-2), 35.3 (C-1), 35.7 (C-20), 36.0 (C-22), 38.4 (C-10), 39.1 (C-6), 39.4 (C-24), 39.7 (C-8), 40.9 (C-12), 42.4 (C-13), 45.1 (C-9), 50.4 (C-14), 55.9 (C-17), 68.4 (C-7), 126.7 (C-4), 168.0 (C-5), 198.9 (C-3). HR-ESI-MS, calcd. for $C_{27}H_{43}O_2$ $[M-H]^-$, 399.3263; found 399.3280.

3.4. X-ray crystal structure determination of 1b

Colorless crystals of **1b** suitable for X-ray analysis were grown by recrystallization from methanol. A crystal having dimensions of 0.25 × 0.25 × 0.30 mm^3 was selected. X-ray intensity data for **1b** were collected on a Rigaku AFC-8 diffractometer equipped with a Mercury CCD detector using monochromated Mo Kα radiation (λ = 0.71070 Å) at 193 K. The structure was solved using direct methods and refined by a full-matrix least-squares procedure based on F^2 using the Crystal Structure crystallographic software package. The crystal structures were refined with anisotropic temperature factors for all non-hydrogen atoms. The positions of hydrogen atoms were generated theoretically, and were refined using the riding model. Crystallographic details and atomic coordinates are summarized in Tables 1 and 2, respectively.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2013.05.011.

References

[1] a Princen GM. Regulation of bile acid biosynthesis. Curr Pharm Des 1997;3:59–84;
b Russell WD. The enzymes, regulation, and genetics of bile acid synthesis. Annu Rev Biochem 2003;72:137–74;
c Norlin M, Wikvall K. Enzymes in the conversion of cholesterol into bile acids. Curr Mol Med 2007;7:199–218;
d Bernhardsson C, Björkhem I, Danielsson H, Wikvall K. 12α-Hydroxylation of 7α-hydroxy-4-cholesten-3-one by a reconstituted system from rat liver microsomes. Biochem Biophys Res Commun 1973;54:1030–8.

[2] Heubi EJ, Setchell DRK, Bove EK. Inborn errors of bile acid metabolism. Liver Dis 2007;27(3):282–94.

[3] a Setchell DRK, Suchy JF, Welsh BM, Zimmer-Nechemias L, Heubi J, Balistreri FW. Δ^4-3-Oxosteroid 5β-reductase deficiency described in identical twins with neonatal hepatitis. J Clin Invest 1988;82:2148–57;
b Shneider LB, Setchell DRK, Whitington FP, Neilson AK, Suchy JF. Δ^4-3-Oxosteroid 5β-reductase deficiency causing neonatal liver failure and hemochromatosis. J Pediatr 1994;124:234–8.

[4] a Sauter G, Berr F, Beuers U, Fischer S, Paumgartner G. Serum concentrations of 7α-hydroxy-4-cholesten-3-one reflect bile acid synthesis in humans. Hepatology 1996;24:123–6;
b Gälman C, Arvidsson I, Angelin B, Rudling M. Monitoring hepatic cholesterol 7α-hydroxylase activity by assay of the stable bile acid intermediate 7α-hydroxy-4-cholesten-3-one in peripheral blood. J Lipid Res 2003;44:859–66.

[5] Wedlake L, A'Hern R, Russell D, Thomas K, Walters RFJ, Andreyev JNH. Systematic review: the prevalence of idiopathic bile acid malabsorption as diagnosed by SeHCAT scanning in patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther 2009;30:707–17.

[6] Alexander LD, Fisher FJ. A convenient synthesis of 7α-hydroxy-cholest-4-en-3-one by the hydroxypropyl-β-cyclodextrin-facilitated cholesterol oxidase oxidation of 3β,7α-cholest-5-ene-3,7-diol. Steroids 1995;60:290–4.

[7] a Labaree D, Hoyte MR, Hochberg BR. A direct steroselective synthesis of 7β-hydroxytestosterone. Steroids 1997;62:482–6;
Li S, Pang J, Wilson KW, Schroepfer Jr JG. Sterol synthesis. Preparation and characterization of fluorinated and deuterated analogs of oxygenated derivatives of cholesterol. Chem Phys Lipids 1999;99:33–71.

[8] a Kuramoto T, Kihira K, Matsumoto N, Hoshita T. Determination of sulfated position in 5β-bufol sulfate by a carbon-13 nuclear magnetic resonance study. Chem Pharm Bull 1981;29:1136–9;
b Babu P, Maitra U. Synthesis and in vitro cholesterol dissolution by 23- and 24-phosphonoblie acids. Steroids 2005;70:681–9.

[9] a Anelli LP, Biffi C, Montanari F, Quici S. Fast and selective oxidation of primary alcohols to aldehydes or to carboxylic acids and secondary alcohols to ketones mediated by oxoammonium salts under two-phase conditions. J Org Chem 1987;52:2559–62;
b de Nooy AJE, Besemer CA, van Bekkum H. On the use of stable organic nitroxyl radicals for the oxidation of primary and secondary alcohols. Synthesis 1996:1153–74;
c Bode WJ, Carreira ME. Stereoselective syntheses of epothilones A and B via nitrile oxide cycloadditions and related studies. J Org Chem 2001;66:6410–24.

[10] Iida T, Nishida S, Chang CF, Niwa T, Goto J, Nambara T. Potential bile acid metabolites. XXI. A new synthesis of allochenodeoxycholic and allocholic acids. Chem Pharm Bull 1993;41(4):763–5.

[11] Hoshita T, Shefer S, Mosbach HE. Conversion of 7α,12α-dihydroxycholest-4-en-3-one to 5α-cholestane-3α,7α,12α-triol by iguana liver microsomes. J Lipid Res 1968;9:237–43.

[12] Nicolaou CK, Montagnon T, Baran SP, Zhong LY. Iodine(V) reagents in organic synthesis. Part 4. *o*-Iodoxybenzoic acid as a chemospecific tool for single electron transfer-based oxidation processes. J Am Chem Soc 2002;124:2245–58.

[13] Li Q, Tochtrop PG. A stereoselective synthesis of the allo-bile acids from the 5β-isomers. Tetrahedron Lett. 2011;52:4137–9.

[14] Frigerio M, Santagostino M, Sputore S. A user-friendly entry to 2-iodoxybenzoic acid (IBX). J Org Chem 1999;64:4537–8.

[15] Marat K, Templeton FJ, Kumar PSV. An NMR study of A-ring conformation in some 4-en-3-one steroids. Magn Reson Chem 1987;25:25–30.

[16] Faucher F, Cantin L, Luu-The V, Labrie F, Breton R. The crystal structure of human Δ4-3-ketosteroid 5β-reductase defines the functional role of the residues of the catalytic tetrad in the steroid double bond reduction mechanism. Biochemistry 2008;47:8261–70.

[17] Une M, Shinonaga Y, Matoba N, Kuroki S, Kihira K, Hoshita T. Identification of new bile alocohols, 5β-cholestane-3α,7α,24,26-tetrol, 5β-cholestane-3α,7α,26,27-tetrol, and 5β-cholestane-3α,7α,26,27-tetrol in human gallbladder bile. J Lipid Res 1986;27:1318–23.

[18] Kannan A, Clercq DE, Pannecouque C, Witvrouw M, Hartman LT, Turpin AJ, et al. Synthesis and anti-HIV activity of a bile acid analog of cosalane. Tetrahedron 2001;57:9385–91.

[19] Dayal B, Batta KA, Shefer S, Tint SG, Salen G, Mosbach HE. Preparation of 24(R)- and 24(S)-5β-cholestane-3α,7α,24-triols and 25(R)- and 25(S)-5β-cholestane-3α,7α,26-triols by a hydroboration procedure. J Lipid Res 1978;19:191–5.

[20] Yamazaki K, Kawahara T, Shimizu K. Metabolic studies of bile acids XXVII partial synthesis of some 3-keto-coprostane derivatives. Yonago Acta Medica 1959;4:37–9.
[21] Berséus O, Danielsson H, Kallner A. Synthesis and metabolism of cholest-4-ene-7α,12α-diol-3-one and 5β-cholestane-7α,12α-diol-3-one. J Biol Chem 1965;240:2396–401.
[22] Takada K, Ōsaka H, Horiki A. Bile acids and steroids. XIX. Sythesis of some of 7-oxygenated 5β-cholestane. Yakugaku Zasshi 1961;81:325–30.
[23] Danielsson H. Synthesis and metabolism of Δ^4-cholestene-7α-ol-3-one. Acta Chem Scand 1961;15:242–8.